Application No.: 10/626,477 2 Docket No.: 564462008100

Amendment to the Claims:

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application: Listing of Claims:

Claim 1 (previously presented): A method for isolating and maintaining a cell isolated from a mixed population of uncultivated cells comprising:

- (a) providing a system comprising a growth column with an inlet and an outlet, and a mechanism for moving or circulating a growth medium through the length of the growth column, wherein the growth column comprises openings at either end of the growth column to allow the culture media to flow through the length of the growth column;
 - (b) providing a culture media;
 - (c) providing an encapsulation composition for making a porous microdroplet;
 - (d) providing a mixed population of uncultivated cells;
- (e) encapsulating in the encapsulation composition at least a single cell from the mixed population of uncultivated cells to create a microenvironment in the porous microdroplet;
- (f) placing the porous microdroplet comprising the encapsulated cell in the growth column; and
- (g) incubating the encapsulated cell in the porous microdroplet in the growth column under conditions allowing the encapsulated cell to survive and be maintained, wherein the conditions comprise flowing the culture medium through the length of the growth column, thereby isolating and maintaining the cell.

Claim 2 (previously presented): The method of claim 1, wherein the mixed population of uncultivated cells comprises a mixed population of uncultivated cells from an environmental sample.

Claim 3 (currently amended): The method of claim 2, wherein the environmental sample is selected from the group consisting of: geothermal fields, hydrothermal fields, acidic soils, sulfotara mud pots, boiling mud pots, pools, hot-springs, geysers, marine actinomycetes, metazoan,

endosymionts, ectosymbionts, tropical soil, temperate soil, arid soil, compost piles, manure piles, marine sediments, freshwater sediments, water concentrates, hypersaline sea ice, super-cooled sea ice, arctic tundra, <u>Sargasso Sargosso</u> sea, open ocean pelagic, marine snow, microbial mats, whale falls, springs, hydrothermal vents, insect and nematode gut microbial communities, plant endophytes, epiphytic water samples, industrial sites and *ex situ* enrichments.

Claim 4 (previously presented): The method of claim 2, wherein the environmental sample is selected from the group consisting of: air, water, sediment, soil and rock, or the mixed population of uncultivated cells comprises a eukaryotic cell, a prokaryotic cell or a myxobacteria cell.

Claim 5 (original): The method of claim 1, wherein the mixed population of uncultivated cells comprises a mixture of materials.

Claim 6 (original): The method of claim 5, wherein the mixture of materials comprises a biological sample, soil or sludge.

Claim 7 (original): The method of claim 6, wherein the biological sample comprises a plant sample, a food sample, a gut sample, a salivary sample, a blood sample, a sweat sample, a urine sample, a spinal fluid sample, a tissue sample, a vaginal swab, a stool sample, an amniotic fluid sample or a buccal mouthwash sample.

Claim 8 (previously presented): The method of claim 1, wherein the encapsidated cell comprises a microorganism.

Claim 9 (previously presented): The method of claim 8, wherein the encapsidated microorganism comprises a protozoan cell, a bacterial cell, a yeast cell, or an archaeal cell, or the encapsidated cell is a plant cell, a mammalian cell, or an insect cell.

Claim 10 (previously presented): The method of claim 1, wherein the cell is an extremophile.

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Claim 11 (previously presented): The method of claim 10, wherein the extremophile cell is an extremophile hyperthermophile cell, a psychrophile cell, a halophile cell, a psychrotrophs cell, an alkalophiles cell, or an acidophiles cell.

Claim 12 (previously presented): The method of claim 1, wherein a single cell is encapsulated in a porous gel microdroplet (GMD).

Claim 13 (original): The method of claim 12, wherein the porous gel microdroplet (GMD) comprises a hydrogel matrix or a selectively permeable membrane.

Claim 14 (previously presented): The method of claim 12, wherein the porous gel microdroplet (GMD) comprises a CELMIXTM emulsion matrix or a CELGELTM encapsulation matrix, or a combination thereof.

Claim 15 (previously presented): The method of claim 12, wherein one cell is encapsulated in each porous gel microdroplet (GMD).

Claim 16 (previously presented): The method of claim 12, wherein one to four cells is encapsulated in each porous gel microdroplet (GMD).

Claim 17 to 20 (canceled)

Claim 21 (original): The method of claim 1, wherein conditions allowing the encapsulated cell to survive and be maintained comprise providing nutrients at *in situ* concentrations.

Claim 22 (currently amended): The method of claim 1, wherein conditions allowing the encapsulated cell to survive and be maintained comprise pumping through through the growth column an aqueous nutrient mixture.

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Claim 23 (previously presented): The method of claim 1, further comprising incubating and culturing the encapsulated cell in the growth column under conditions allowing growth or proliferation of the cell into a microcolony comprising at least two daughter cells.

Claim 24 (original): The method of claim 23, wherein the microcolony comprises between about 4 and 100 cells.

Claim 25 (original): The method of claim 1, further comprising isolating a gel microdroplet.

Claim 26 (previously presented): The method of claim 23, further comprising isolating a gel microdroplet and isolating a microcolony from the gel microdroplet.

Claim 27 (previously presented): The method of claim 26, further comprising isolating a cell from the isolated microcolony.

Claim 28 (previously presented): The method of claim 26, wherein isolating the gel microdroplet comprises sorting the encapsulated microcolony by size.

Claim 29 (previously presented): The method of claim 28, wherein sorting the encapsulated microcolony by size comprises using a flow cytometry.

Claim 30 (previously presented): The method of claim 25, wherein the gel microdroplet is isolated by a fluorescence activated cell sorting (FACS) or by a capillary array-based system.

Claim 31 (original): The method of claim 27, further comprising maintaining the isolated cell by re-encapsulating and re-culturing the isolated cell.

Claim 32 (original): The method of claim 31, wherein between about 20 and 100 cells are maintained in each re-encapsulated microcolony.

Claim 33 (original): The method of claim 31, further comprising screening the interactions between encapsulated cells.

Claim 34 (original): The method of claim 25, further comprising re-culturing the isolated gel microdroplet under the same or different conditions.

Claim 35 (original): The method of claim 1, further comprising direct amplification of nucleic acid from the encapsulated cell.

Claim 36 (previously presented): The method of claim 23, further comprising direct amplification of nucleic acid from the cultured encapsulated cells.

Claims 37 to 38 (canceled)

Claim 39 (previously presented): A method for isolating a cell from a mixed population of uncultivated cells comprising:

- (a) providing a system comprising a growth column with an inlet and an outlet, and a mechanism for moving or circulating a growth medium through the length of the column, wherein the growth column comprises openings at either end of the column to allow the culture media to flow through the length of the column;
 - (b) providing a culture media;
 - (c) providing an encapsulation composition for making porous microdroplets;
 - (d) providing a mixed population of uncultivated cells;
- (e) encapsulating in the encapsulation composition at least a single cell from the mixed population of uncultivated cells to create a microenvironment in the porous microdroplet;
- (f) placing the porous microdroplet comprising the encapsulated cell in the growth column; and
- (g) incubating the encapsulated cell in the porous microdroplet in the growth column under conditions allowing the encapsulated cell to survive, wherein the conditions comprise flowing the culture medium through the length of the growth column, thereby isolating the cell.

Claim 40 (previously presented): A method for maintaining a cell isolated from a mixed population of uncultivated cells comprising:

- (a) providing a system comprising a growth column with an inlet and an outlet, and a mechanism for moving or circulating a growth medium through the length of the column, wherein the growth column comprises openings at either end of the column to allow the culture media to flow through the length of the column;
 - (b) providing a culture media;
 - (c) providing an encapsulation composition for making porous microdroplets;
 - (d) providing a mixed population of uncultivated cells;
- (e) encapsulating in the encapsulation composition at least a single cell from the mixed population of uncultivated cells to create a microenvironment in the porous microdroplet;
- (f) placing the porous microdroplet comprising the encapsulated cell in the growth column; and
- (g) incubating the encapsulated cell in the porous microdroplet in the growth column under conditions allowing the encapsulated cell to survive and be maintained, wherein the conditions comprise flowing the culture medium through the length of the growth column, thereby maintaining the cell.

Claim 41 (previously presented): The method of claim 1, wherein the growth media comprises an amino acid supplemented medium; an organic rich medium diluted in seawater; a marine medium; a seawater amended with a mixture of amino acids; a seawater amended with inorganic nutrients; a sterile filtered seawater; a diluted soil extract, or a combination thereof.

Claim 42 (previously presented): The method of claim 1, wherein the encapsulated cells are incubated in the porous microdroplet in the growth column for up to five weeks, or at least for between three hours and five weeks, or from about between 20 minutes and several weeks or months.

Claim 43 (previously presented): The method of claim 10, wherein the cells comprise bacterioplankton, *Planctomycetales*, *Planctomycetes*, or *Cytophaga*, or *Lavobacterium*, or *Bacteroides*, or *Proteobacteria*, or *Salinibacter*, or *Rhodothermus*, or *Methanococcus*.

Claim 44 (previously presented): The method of claim 1, wherein the growth column further comprises a filter membrane at the inlet and the outlet.

Claim 45 (previously presented): The method of claim 1, wherein the growth column further comprises a media reservoir.

Claim 46 (previously presented): The method of claim 45, wherein the growth column further comprises a filter membrane at the inlet and the outlet, and the filters prevent free-living cells from contaminating the media reservoir.

Claim 47 (previously presented): The method of claim 1, wherein the growth column further comprises a filter membrane at the inlet and the outlet, and the filters retain the porous microdroplets in the growth column while allowing non-encapsulated cells to be washed out of the growth column by the flow of the growth medium.